ligands to unbound aptamer, so as to recover a second sample of aptamer bound to target molecules; and

(d) using a quantitative replicative procedure comprising a replicative polymerase reaction to determine a quantity of aptamer specific for each target molecule in the second sample and therefore related to the concentration of target molecule in the first sample.

Claims 35-44. Cancelled.

Remarks

Amendments to the Specification

The Examiner pointed out an error in the application text, relative to Fig. 1. Figure 1 properly indicates that elements X and Y are assigned numbers **102** and **103**, respectively, in contrast to the clearly erroneous assignment of numbers **103** and **104**, respectively, in the text of the specification on pp. 20-21.

Amendments to the Claims

Claims 1, 2, 29-33, and claim 45 are herein amended. Claims 35-44 were cancelled previously in Response A, with Applicants reserving the right to prosecute these claims at a later date.

Claims 1 and 45 are herein amended to add the limitation "comprising a replicative polymerase reaction" to step (d) of the method for "... quantitatively assaying one or more target molecules ..." to more clearly distinguish to presently claimed invention from the prior art. Support for this application is found throughout the specification, and in original claims 20 and 45.

Claim 2 is amended to clarify whether the phrase "natural or synthetic" was intended to modify all nucleic acid aptamers, or just single-stranded aptamers, by deleting the phrase.

Claims 29-31 are herein amended to replace the phrase "quantitative replicative technique" with the phrase "quantitative replicative *procedure*, for reasons of consistency

and clarity." These amendments make claims 29-31 more consistent, and more complete, and provide these claims with proper antecedent bases. Support for these amendments is found in original claims 1, 20, and 45, among other places.

Multiple-dependent claims 32 and 33 are herein amended to properly depend from "any one of claims 30 *or* 31" in contrast to the original language for these claims which were impermissibly directed to "A method according to claims 30 *and* 31..."

Objections to the specification and to claims 1, 45, and claim 29

Applicants would like to point out that the specification at p. 21, line 4 was previously amended in Response C, filed September 23, 2002, to delete "**103** and **104**" and insert "**102** and **103**". However, in case that amendment was not entered, or executed properly (since the entire paragraph was not replaced, but merely that phrase on p. 21, line 4), Applicants have chosen to again amend the specification to correct this error. Thus, the paragraph that spans the bottom of p.20 and continues onto the top of p. 21, which discusses elements **X** and **Y** of Fig. 1, has been amended by deleting the erroneous paragraph which refer to these elements as **103** and **104**, respectively, and by inserting a new paragraph correctly identifying elements X and **Y** as elements **102** and **103**, respectively. Therefore, Applicants respectfully request withdrawal of the objection to the specification.

Claims 1 and 45 have been amended to insert the word "the" between "with" and "aptamer" on lines 6 and 5, respectively. Claim 29 has been amended to insert the word "and" between "aliquots" and "a first" on line 2. Although Applicants do not believe the claims, as written, were problematic, Applicants agreeably made the corrections to claims 1 and 45, and claim 29, as requested by the Examiner. As such, Applicants respecfully request withdrawal of the objections to claims 1 and 45 and claim 29.

Rejections based on 35 U.S.C. § 112, para. 2 - Indefiniteness

Claims 1-34 and 45 were rejected as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention

(see Office Action, p. 3) but no reason for this rejection is made.

The Office Action states that the phrase "natural or synthetic" in claim 2 is vague and indefinite, as it is not clear whether the phrase modifies just single-stranded DNA or all of the examples of nucleic acids which follow. Applicants have deleted this phrase from claim 2. As such, Applicants respectfully submit that claim 2, indeed, all of the pending claims, are definite. Reconsideration of the claims and withdrawal of the indefiniteness rejections under 35 U.S.C. § 112, para. 2 is therefore requested.

Prior art rejections under 35. U.S.C. § 103 (a)

The rejection of claims 1-2, 6-9, 13-14, 16-18, 20-24, 33 and 45, for obviousness, depends on combinations of Li (US6,180,348(B1)) with Bièche et al. (*Int J Cancer*:**78**,661-666 (1998)) - hereinafter "Bièche" - and the rejections of the remaining claims depend on combinations of Li and Bièche in view of various other references.

Therefore, Applicants will address the rejections based on Li and Bièche, given that all remaining rejections involve combinations of art which rely on these two references.

Li has been cited as an example of a prior art reference which discloses generally all of the elements of claim 1, except the requirement for a replicative procedure that is quantitative and which measures the amount of aptamer by denaturing the aptamer from the target ligand, adding primers and determining a number of replicative cycles. See and Office Action, p. 5, and first two lines of p. 6. In other words, Li is cited as the "aptamer" reference, analogous to, but even less on point than, the citation of Griffin et al (US5,756,291) as the "aptamer reference" in, for example, the previous office action of September 24, 2003.

Bièche has been combined with Li because according to the Office Action, Bièche teaches a real-time quantitative PCR ("qPCR") detection. Thus, Bièche is the "PCR" reference combined with Li to support an obviousness rejection (see *id.*, p. 6 first and second para) analogous to the Jayasenu reference (US5,989,823) cited in previous office actions in combination with Griffin. Moreover, the present Office Action adds nothing to the previous combination of Griffin and Jayasenu to support an obviousness rejection.

And, as explained repeatedly in previous responses and telephone interviews, the claimed subject matter in the instant application is not merely a combination of how to generate aptamers highly specific to a target molecule with the use of qPCR to quantitate the amount of target molecule originally present in a sample.

As argued in Responses D (July 11, 2003) and E (November 24, 2003) and re-stated at least in the telephone interview of October 8, 2003, and further as herein amended, the presently claimed invention requires "using a quantitative replicative procedure comprising *a quantitative replicative polymerase reaction* to determine a *quantity of aptamer* specific for each target molecule." See e.g. claims 1 and 45, emphasis added, and specification at p. 5, lines 22-23. Claims 1 and 45 are methods for "quantitatively assaying one or more target molecules in a first sample..." not, as disclosed in Li, "methods of preparing oligonucleotide libraries, isolating oligonucleotide aptamers to target molecules from the libraries, and using aptamers to purify target molecules by affinity separation." See Li, Abstract, and throughout. The Office Action states that Li discloses all of the elements of the instantly claimed invention except for the use of a quantitative replicative procedure to quantitate the amount of aptamer bound to target molecule, and this amount of target molecule. See Office Action, p. 5 to top of p. 6. The Examiner has completely mixed and matched the various procedures disclosed in Li to support an erroneous conclusion that Li discloses all the elements in claim 1 except use of qPCR. Claim 1 of the instant application starts where Li leaves off, in that a preparation of aptamers previously identified as specific for target molecules is added to a first sample, the aptamers are allowed to bind to the target molecules in the sample, and then any remaining unbound aptamer is separated from bound aptamer by use of immobilized ligands on, for example, a column, such that a second sample containing only aptamer bound to target molecule is obtained, after which a quantitative replicative procedure is used to quantify the amount of bound ligand, giving a direct measurement of the amount of target molecule present.

Nowhere in Li is there any disclose of a separation of unbound aptamer from aptamer bound to target molecule using immobilized ligand, and never is there any quantitative determination of aptamer bound directly to target molecule. The Office Action states that column 10, lines 38-50 disclose aptamers binding to immobilize

ligands, and then states that col. 7, lines 49-51 disclose that PCR can be used to detect and quantitate target molecules. This is a total mis-application of what is disclosed in Li relative to the instantly claimed subject matter. In col. 10, lines 38-50, Li discloses how to prepare an oligonucleotide library of aptamers bound to a solid support (see Heading for Example 3) in the context of using such a library to purify a desired protein. In contrast, claims 1 and 45 of the instant application require that *unbound aptamers* in a sample containing unbound aptamers and aptamers bound to a target molecule *are separated* by addition of the sample to immobilized ligands *which bind the unbound aptamer*, allowing the bound aptamer to flow through and be separated.

Nowhere does Li disclose use of immobilized ligands that are not aptamers to separate unbound aptamers from aptamers bound to a target molecule in a sample containing both. Adding to the faulty analysis, the Office Action states immediately after this mis-application of what Li discloses in col. 10, that PCR can be used to detect and quantitate target molecule as disclosed by Li in col. 7, lines 49-51. In col. 7, Li is disclosing how one can take target molecule, immobilized to a solid support, *to identify highly selective aptamers* to that target molecule, by amplifying and then isolating and sequencing the aptamer which binds to the immobilized target molecule. In contrast, claims 1 and 45 of the instant application start with the assumption that highly specific aptamers have already been identified. None of the claims of the instant application, or elements of the claims, is directed to the methodology disclosed by Li in col. 7. This section of Li is not at all about using the oligomer-bound resins/beads/solid supports discussed in col. 10, Example 3 to separate unbound aptamers from aptamers bound to a low abundance target molecule in a sample, to quantify how much target molecule is present. Col. 7 of Li is simply disclosing how one can use immobilized target molecules to identify highly specific aptamers to that target molecule by amplifying oligomers that bind to such immobilized target molecules and sequencing them to see what they are.

Nowhere at all in Li is there any contemplation or suggestion that immobilized ligands can be used *to separate unbound aptamers from aptamers bound to a target molecule* in a sample containing target molecules, or any contemplation or suggestion that aptamers can be used to directly quantify the amount of a low-abundance target molecule present in a sample. Moreover, it is contrary to the purpose of Li to use aptamers to

quantify a low abundance target molecule since the whole point of Li is to generate libraries of oligonucleotides, use immobilized target molecule to identify highly specific aptamers to such target molecule as an alternative to the previously disclosed, and patented, SELEX method of Griffin, and then use such identified aptamers to fish out desired target molecules from new sources/organisms/samples where that target molecule has not been previously found.

In light of the inappropriateness of citing Li as disclosing all the elements of the presently claimed subject matter except use of a replicative procedure to quantify aptamer which binds to a target molecule, the combination of Li with Bièche, adds nothing to support an obviousness rejection. There can be no simple combination of an "aptamer" reference with a "qPCR" reference, as explained to previous Examiner Cook and supervisory Examiner Le on several occasions, because the claimed subject matter of the instant application is not a simple combination of aptamer technology with qPCR technology, but is rather application of aptamer technology in a way never before imagined, combined with the power of quantitative replicative procedures, to arrive at a novel, totally unprecedented methodology for directly quantitating the amount of low abundance target molecule in a sample. The presently claimed subject matter uses aptamers highly selective for the desired target molecule, coupled with a separation step to isolate only aptamer bound to target molecule, followed by a quantitative replicative procedure, to directly quantitate the amount of target molecule in a sample.

The presently claimed subject matter is the epitome of inventiveness. Aptamer technology has been adapted for use with a quantitatively replicative procedure to allow direct quantitation of a target molecule in a sample solution. As disclosed and claimed in the instant application, aptamer technology is used in a manner never before contemplated, and coupled with a quantitative replicative procedure to directly quantitate aptamer bound in a one-to-one complex with target molecule that has been separated from all unbound aptamer so that the quantitative replicative procedure provides a direct measurement of the amount of target molecule present in the sample.

As stated in MPEP §2142, a *prima facie* case of obviousness requires three elements. First all elements of the presently claimed invention must be disclosed or suggested in the cited prior art. Second, there must be a suggestion to make the

combination of references or to modify the reference(s), in the art itself or the knowledge generally available in the field. And third, there must be an expectation of success. In the present case, the *prima facie* case fails on two of the three criteria - lack of motivation, and disclosure/teaching/suggestion of every element in the claims.

First, in the present case, the cited references, alone or in combination, do not disclose all the elements of the claimed invention. There is no disclosure in Li, alone or combined with Bièche, to use immobilized ligands to separate unbound aptamer from aptamer bound to target molecule. Neither is there any disclosure in Li, alone or combined with Bièche to use a quantitative replicative procedure to amply aptamers directly bound to target molecules that have been separated from unbound aptamers, to directly quantitate the amount of target molecule in a sample.

Moreover, MPEP § 2145, subsection X. A. holds that impermissible hindsight cannot be used to support an obviousness rejection when the knowledge required to modify the cited references to arrive at the claimed invention comes from the Applicant's own disclosure. Although those skilled in the art of qPCR may understand that the technique could be used to amply either a target molecule or an aptamer bound to a target molecule, there is no suggestion in any reference, cited in this Office Action or previous Office Actions, to modify the normal use of aptamer technology - i.e. - as a qualitative tool for fishing out molecules/proteins from a source that is not known to contain that target molecule - and to add a purification step using immobilized ligands to separate unbound aptamer from bound aptamer, to successfully arrive at the presently claimed invention that is a unique way to quantitate low abundance target molecules in a sample.

Before the instant invention, aptamer technology was limited to methods for identifying aptamers that have higher selectivity to a given target molecule more easily, quickly, and then to methods for using these highly selective aptamers to fish out target molecules what such target molecules had not yet been found. Similarly, qPCR technology was limited to amplifying and quantitating low abundance genes, i.e., the target molecule itself, rather than amplifying and quantitating aptamer bound to target molecule (which may be many types of molecules other than a gene) as a method for directly quantitating the amount of target molecule in a sample.

Without hindsight, there simply is no suggestion in either the references

themselves or the knowledge generally available in the art, to modify or combine the references to arrive at the present invention. Applicants have creatively and imaginatively taken two known technologies, applied them each in a way not contemplated or done previously, and further combined them in a way not previously contemplated or done, to arrive at the presently claimed subject matter. This represents inventiveness at its very best - to take what people know, and think outside the box for how to use it or apply it in novel ways to solve new problems. Without the impermissible advantage of hindsight, no suggestion to modify *and* combine aptamer technology with a quantitative replicative procedure exists in the art or cited references that would lead someone skilled in the art to the presently claimed invention.

And finally, there is also no expectation of success, given that Li, the "aptamer" reference, states in the Abstract the disclosed methods are to producing libraries of aptamers, using such libraries to isolate aptamers highly selective for desired target molecules, and then using such isolated aptamers to fish out target molecules from novel sources.

Since the remaining references all rely on the combination of Li and Bièche, there is no need to specifically address them beyond what has already been said regarding the basic combination. For at least these reasons, Applicants respectfully submit that the pending claims are not obvious. Therefore, Applicants request reconsideration of the claims and withdrawal of the obviousness rejections.**Conclusion**

Applicants herein petition for a three-month extension of time. A check for $510 is enclosed, to cover the fee for the three-month extension. It is believed that no additional fees are needed for submission of this communication; however, if any additional fees are required for the timely consideration of this application, please charge deposit account number 19-4972.

It is submitted that all of the specification objections and claim objections and rejections have been addressed, and that all of the pending claims are now in a condition for allowance. Accordingly, Applicants respectfully request reconsideration of the application and issuance of a notice of allowance. The Examiner is requested to telephone the undersigned if any matters remain outstanding so that they may be resolved

expeditiously.

Respectfully submitted,

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